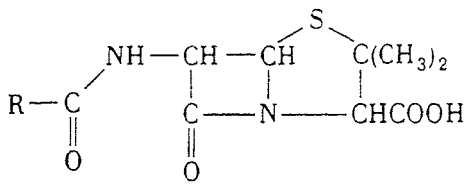
ANTIBIOTICS. The term antibiotic is a broad one, defined by Waksman[34] as "a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms by the action of very small amounts of the antibiotics." Approximately 3000 substances come under this classification,[35] but only about 70 have the necessary combination of patient safety, antimicrobial action, and stability. Penicillins, erythromycin, tetracycline, and cephalosporins are among the most widely used. See Table 6.2. Synthetic modifications of the naturally occurring antibiotic compounds have produced many variations that have clinically superior properties.

Although many antibiotics are now produced commercially, processes with flowcharts for the isolation of three representative ones are shown in Fig. 6.5. Antibiotics have presented many problems to the chemist, the microbiologist, and the chemical engineer since these compounds are often unstable to heat, wide pH ranges, and enzymatic action, and are often decomposed in solution.

PENICILLIN.[36] A number of penicillins, differing only in the composition of the R group, have been isolated from natural media, and hundreds have been semisynthesized. Penicillin G USP, with benzyl for R, generally the most clinically desirable, is the type commercially available, usually combined in salt form with procaine or potassium.



Not only was penicillin the first antibiotic to be produced for widespread use, but it is important in quantities made and in general usefulness. It is practically nontoxic and is one of the most active antimicrobial agents known. Hypersensitivity reactions occur in about 10% of patients. It is also useful in animal feeds to promote growth. It is a tribute to the pharmaceutical industry that the price of penicillin has been reduced from the initial price of $25,000 per gram to $35 per kilogram for procaine penicillin, with a production of over 2950 t in 1981.

It is possible to assist the synthesis of a desired penicillin by supplying the appropriate precursor[37] to the culture, i.e., the acid of the side chain which appears in the amide linkage with 6-aminopenicillanic acid in the final product. The mold preferentially incorporates the precursor added into the corresponding penicillin to the relative exclusion of other precursors present in or formed from the nutrient raw materials in the medium. Thus, to produce benzylpenicillin, the precursor phenylacetic acid is used. Several hundred modifications of the penicillin molecule not found in nature have been made by altering the side chain (R) by use

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[34]Waksman, An Institute of Microbiology: Its Aims and Purposes, *Science* **110** (27) 839, (1949).

[35]Buyske, Drugs from Nature, *CHEMTECH* 5 (6) 361 (1975).

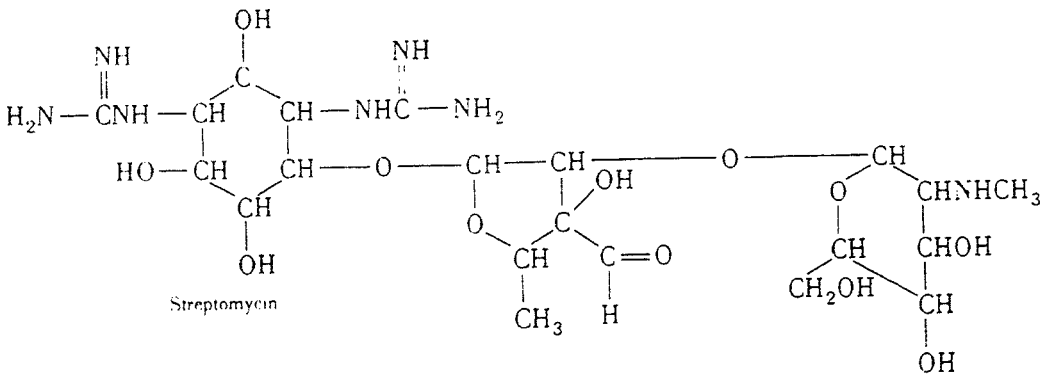
[36]RPS XVI, p. 1135; ECT, 3d ed., vol. 2, 1979, p. 809.

[37]A precursor is defined in the antibiotic industry as a chemical substance that can be incorporated into an antibiotic. The precursor may be used in such a way that an antibiotic modification not normally made by the organism is produced.

of the appropriate precursor in the fermentation.[38] An important modification is penicillin V (V-cillin K, Lilly, potassium phenoxymethyl penicillin), which is produced biosynthetically by using phenoxyacetic acid as a precursor (Fig. 6.5).

ERYTHROMYCIN. Erythromycin USP is, like penicillin, also isolated by solvent-extraction methods. It is an organic base, and extractable with amyl acetate or other organic solvents under basic conditions rather than the acidic ones which favor penicillin extraction.

STREPTOMYCIN.[39] The commercial method for producing this compound is also aerobic submerged fermentation, as outlined in Fig. 6.5 and the appended description. Its formula is



Streptomycin

The structure of streptomycin indicates its highly hydrophilic nature, and it cannot be extracted by normal solvent procedures. Because of the strong-base characteristics of the two substituted guanidine groups, it may be treated as a cation and removed from the filtered solution by ion-exchange techniques.

CEPHALOSPORINS.[40] Cephalosporins were marketed in the late 1950s when penicillin-fast organisms became prevalent. Later, chemically modified cephalosporins were developed to broaden the scope of their use and to be used where the originals were losing their effectiveness. At present, several third generation cephalosporins are awaiting Food and Drug Administration approval. Some of these are already on the market in Europe. Claims are made that these antibiotics are so broad in their activity that they can be used to treat patients before the infectious agent has been identified.

PRODUCTION AND ISOLATION OF PENICILLIN, ERYTHROMYCIN, AND STREPTOMYCIN. Figure 6.5 illustrates how these three important antibiotics of different chemical structure are formed by the life processes of three different microorganisms growing in individual pure cultures and how each one is extracted and purified. The steps in Fig. 6.5 may be

[38]Behrens et al., U.S. Patent 2,562,410 (1951).

[39]RPS XVI, p. 1127.

[40]Third-Generation Antiobiotics Join the Fray, *Chem. Week* 129 (8) 42 (1981).

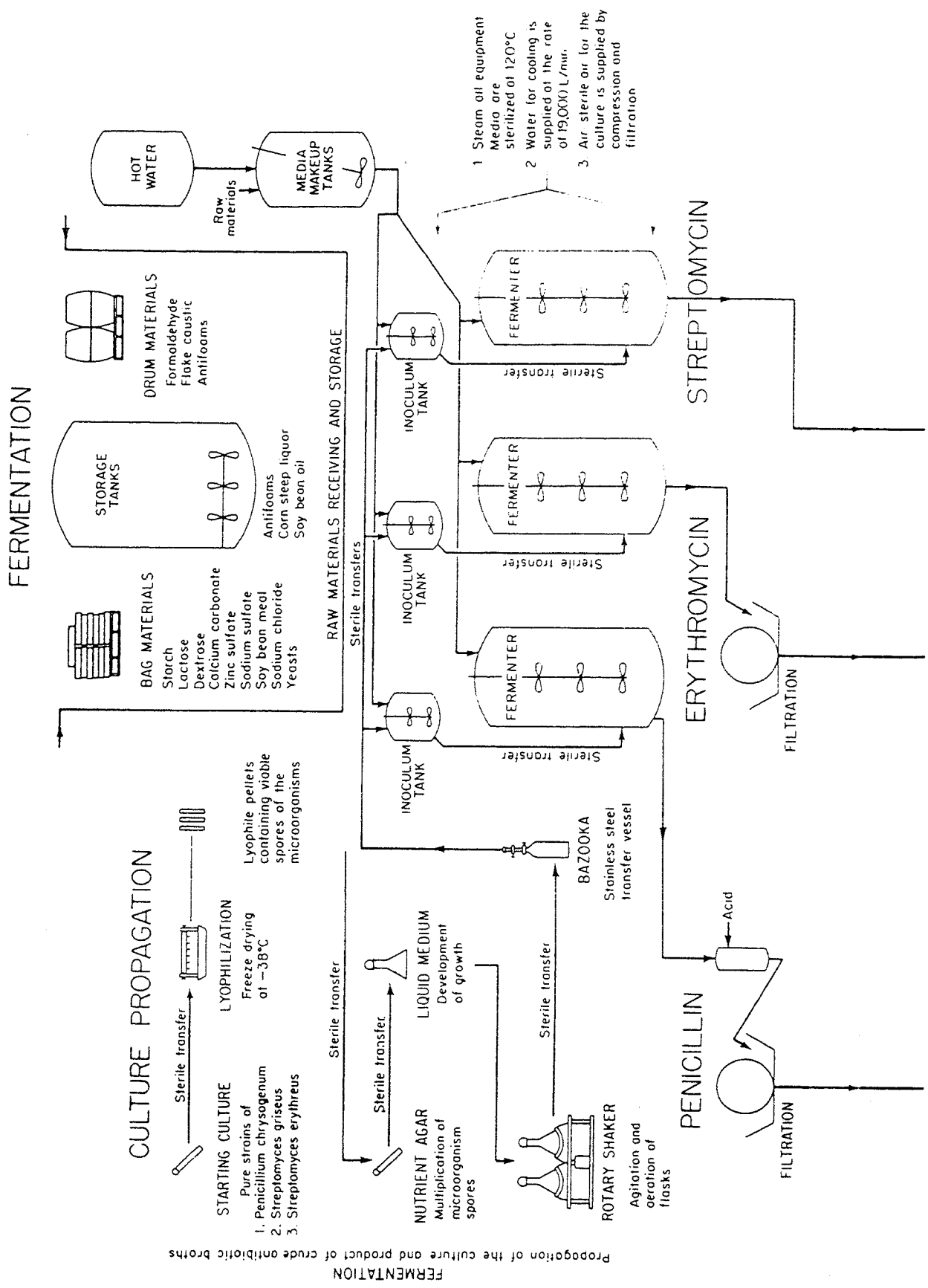
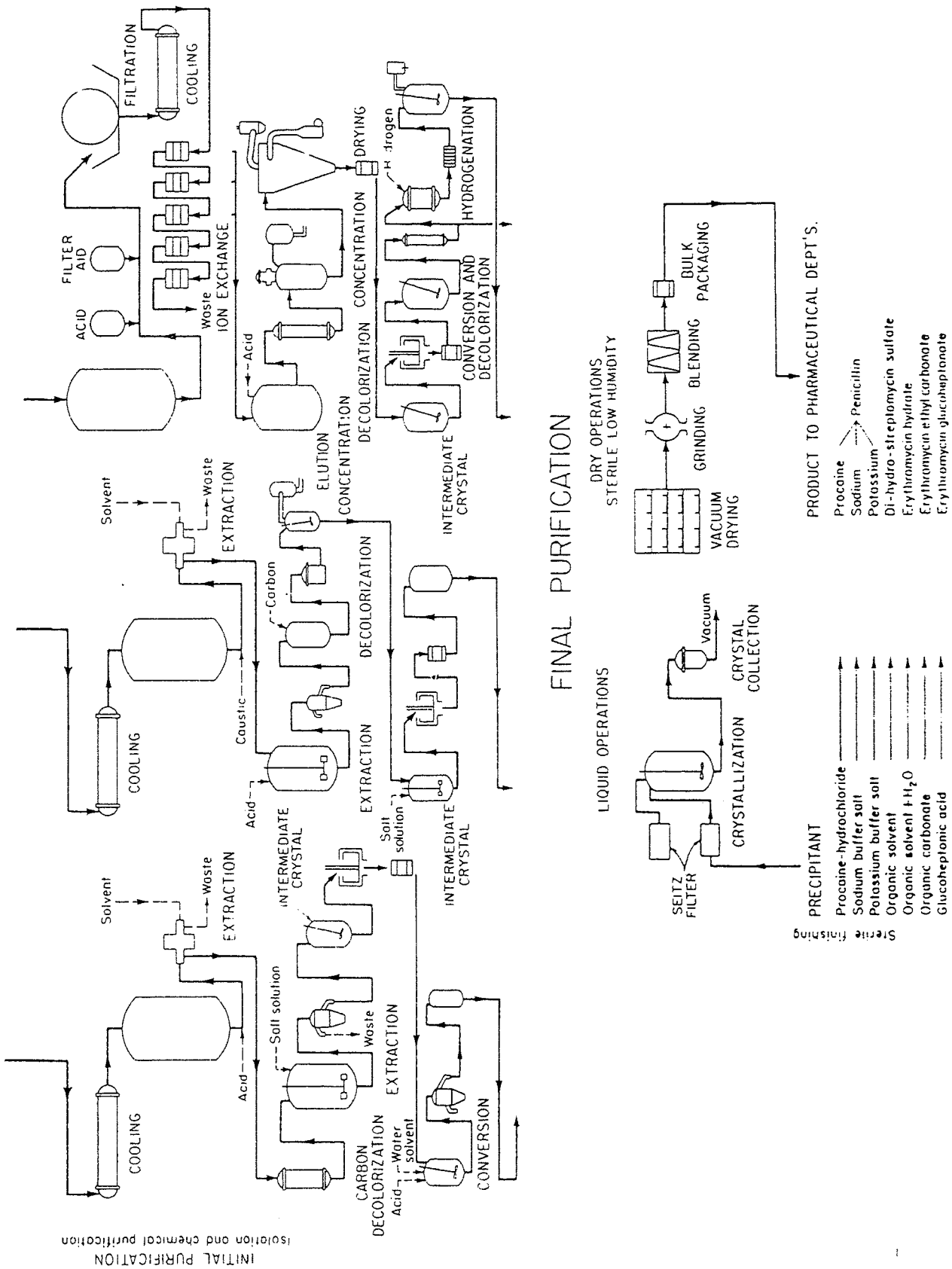


Fig. 6.5. Flowchart for the manufacture of antibiotics by fermentation, purification, extraction, and crystallization for penicillin and erythromycin, and with ion exchange for streptomycin. *(Eli Lilly & Co.)* Typical penicillin fermentation, approximate batch for 45,000-L fermenter (maintain pH preferably below 6.5 and not above 7 and temperature at 25 to 27°C):

Water	37,850 L	Precursor	500 kg
Sugar	3990 kg	Minerals	50 kg
Corn-steep liquor	500 kg	Time	100–150 h



For purification and isolation, 1800 kg of filter aid, 3785 L of amyl acetate, and 150 kg of procaine HCl are required. The yield of procaine benzylpenicillin (procaine penicillin G) is approximately 300 kg.

separated into the following coordinated sequences of *chemical conversions:*

Isolate and purify the required pure, active culture of the respective antibiotic from the starting culture on the slant through to the inoculum tank: for the culture of penicillin, *Penicillium chrysogesium;* of erythromycin, *Streptomyces erythreus;* of streptomycin, *Streptomyces griseus.*

Prepare and steam-sterilize at 120°C the fermentation media containing proteins, carbohydrates, lipids, and minerals.

Introduce the inoculum into the large, stirred, air-agitated fermenters to facilitate rapid metabolism. The air must be sterile.

Ferment, or "grow," the respective microorganism under conditions favorable to the planned antibiotic. Time, 100 to 150 h at 25 to 27°C.

Filter off the mycelium (cells and insoluble metabolic products) of the respective microorganism and wash, using a string discharge continuous rotary filter. Dry (sell for fertilizer).

The filtrates from the respective microorganism contain the antibiotic which must be *initially* purified and separated, and *finally* purified according to the properties of the respective antibiotic, as in the following outline.

For penicillin the purity is only 5 to 10%, impurities being medium components and metabolic products of the mold. Concentration is from 5 to 10 mg/ml.

Penicillin[41]

Cool the aqueous solution and acidify (H_2SO_4) to a pH of 2.0, resulting in an acid (and extractable) form of penicillin.

Solvent-extract in a Podbielniak countercurrent rotating contactor (Fig. 40.6) using a 1:10 volume of amyl acetate, giving a penicillin purity 75 to 80%.

Reverse the extraction at a pH of 7.5 into an aqueous solution with enhanced concentration and purity. Carbon-decolorize; add salt solution; quickly centrifuge to remove slime.

Stir to form intermediate crystal.

Centrifuge to recover intermediate crystal.

Recover values from the mother liquor by a countercurrent acidulated solvent (amyl acetate).

In the final purification, sterilize through Seitz filters.

Precipitate, e.g., with procaine HCl, to obtain procaine penicillin or as potassium or sodium salt.

Erythromycin[42]

Alkalize (NaOH) and solvent-extract (amyl acetate) in a Podbielniak countercurrent extractor.

Add acid and centrifugate the slime.

Decolorize with carbon.

Salt out the intermediate crystals and centrifuge.

Recrystalize or precipitate to obtain erythromycin.

Sterilize through Seitz filters.

Dry under sterile conditions.

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[41]Flowcharts for Penicillin, *Chem. Eng.* 64 (5) 247 (1957); Lowenheim and Moran, *Industrial Chemicals*, 4th ed., Wiley-Interscience, New York, 1975, p. 589.

[42]Hamel, *Antibiot. Chemother.* 11 328 (1961).

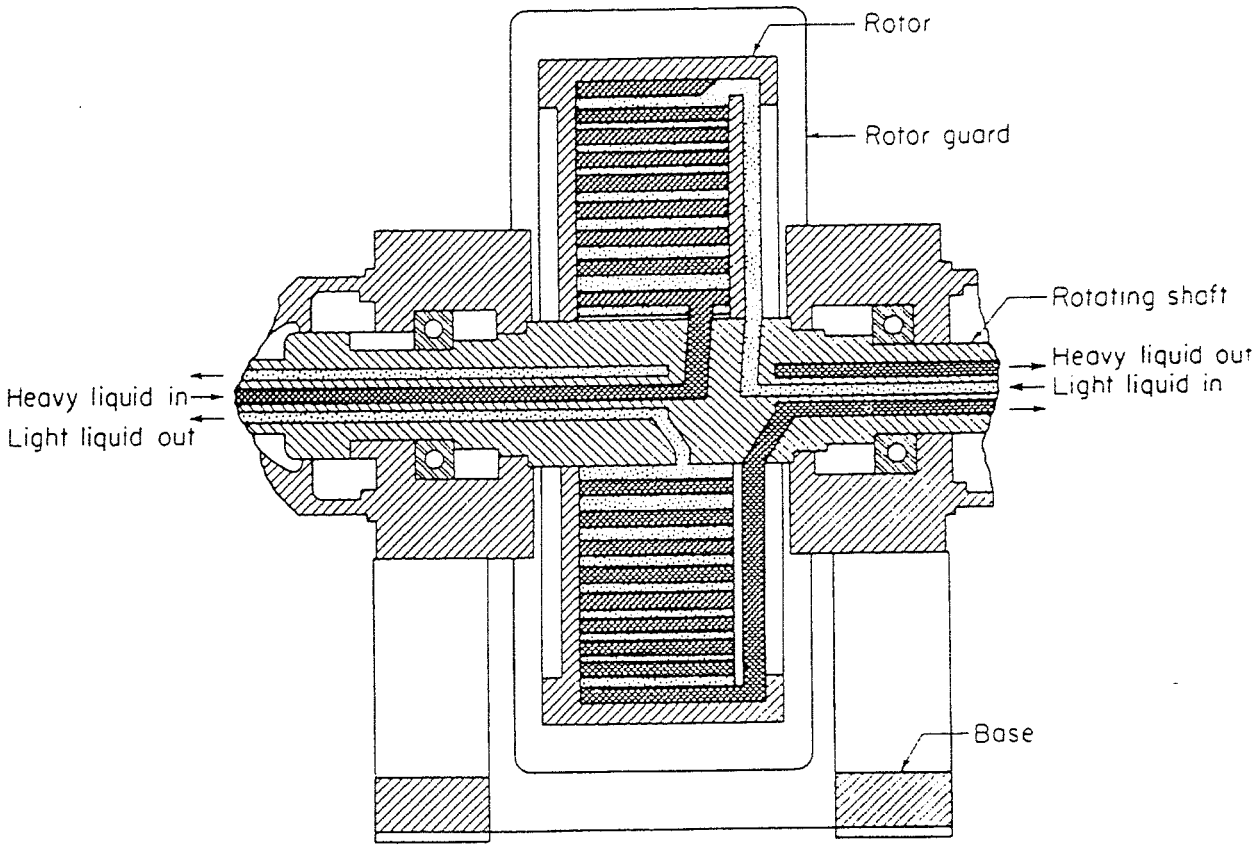


Fig. 6.6. The Podbielniak, or centrifugal, refiner, contactor, and separator. The liquids are introduced through the rotating shaft. The light liquid is led internally to the drum periphery and then out through the axis. The heavy liquid is led through the rotating shaft to the axis of the drum and out from the drum periphery. Rapid rotation causes a radial counterflow of the two liquids. [*See Perry, pp. 21-29; Todd and Podbielniak, Centrifugal Extraction, Chem. Eng. Prog.* 61 *(5) 69 (1965).*]

Streptomycin

Extract streptomycin from the aqueous "beer" with concentrations of 10 to 15 mg/ml by ion-exchange resin (Rohm & Haas IRC-50, carboxylic acid cation exchanger).

Elute from the resin with acidulated water.

Concentrate, decolorize, crystallize, and centrifuge the intermediate crystals.

Convert to desired salt and sterilize through a Seitz filter.

Dry under sterile conditions.

BIOLOGICALS

"Biologic product[43] means any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment, or cure of diseases or injuries to man." They arise from the action of microorganisms, and they are used for prophylaxis, treatment, and diagnosis of infections and allergic diseases. Their significance greatly increased with the introduction of Salk and Asian flu vaccines, followed by oral vaccines for poliomyelitis and the various types of measles vaccines. Biological products are valuable for producing immunity to infections and preventing epidemics of contagious diseases.

[43]RPS XVI, p. 1315 and chaps. 72-74 for further information.

STEROID HORMONES[44]

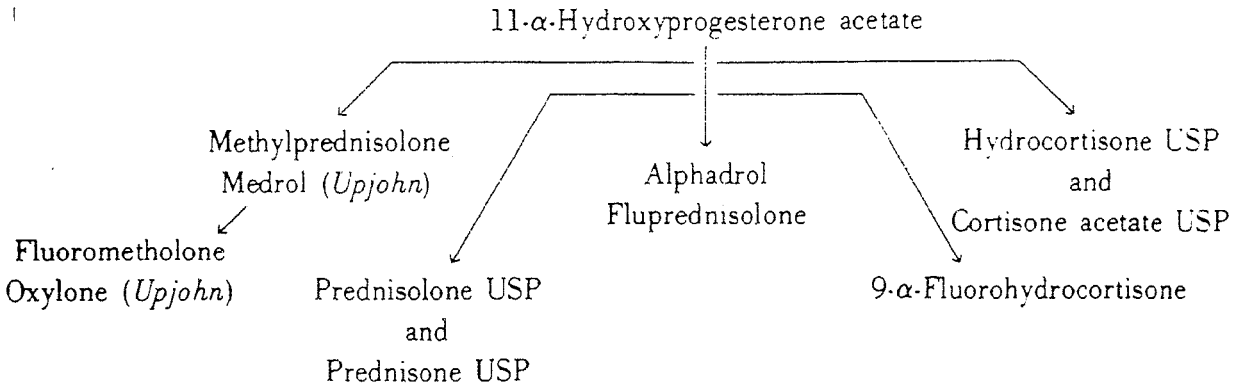
The annual value of bulk steroids produced in the United States increases continually. Steroids[45] used in medicine may be divided into the major classes of corticoids, anabolic androgens, progestational hormones, and estrogenic hormones. Most of those sold and used in medicine today are produced synthetically and are not naturally occurring steroids at all, but chemical modifications of them. There are, for example, at least 16 different so-called corticoids on the market (not counting their various esters and salts), only two of which—cortisone and hydrocortisone—are identical with those found in nature.

In medicine corticoids are perhaps more widely used than steroids of other types. They are used particularly in rheumatic diseases, in inflammatory conditions of the skin, and in allergic conditions. Other uses are in menstrual irregularities, menopause, and control of fertility and conception. There are many other indications for the use of steroids in medicine: in renal and cardiovascular diseases, in certain types of cancer, and in various types of stress reactions.

OUTLINE OF SYNTHESIS FROM SOYA, WHICH CONTAINS THE STEROID NUCLEUS:

$$\text{Crude soya sterols} \longrightarrow \text{stigmasterol} \xrightarrow[\text{steps}]{\text{chemical}^{46}} \text{progesterone}$$

Progesterone is converted[47] in high yield to the key intermediate for most of these sequences. 11-α-hydroxyprogesterone, by aerobic fermentation with a mold, such as *Rhizopus arrhizus*. Another fermentation step which enters into the synthesis of Medrol, Alphadrol, Oxylone, and other steroids, which have a double bond between C_1 and C_2, is dehydrogenation with a *Septomyxa* species. The following chart depicts briefly the various directions in which the key intermediate may be carried.



Other commercial processes for steroid hormone syntheses are based on bile acids and the plant steroid diosgenin obtained from a Mexican yam.

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[44]Courtesy of the Upjohn Co. See also RPS XVI, pp. 392, 902 ff.

[45]Applezweig, *Steroid Drugs*, vol. 1, McGraw-Hill, New York, 1962; vol. 2, Holden-Day, San Francisco, 1964.

[46]U.S. Patent 3,005,834; Lednicer and Mitscher, op. cit., p. 159.

[47]U.S. Patents 2,602,769; 2,735,800; 2,666,070.

VITAMINS

For *vitamins*, the U.S. Tariff Commission reports a total production of 19×10^3 t for 1981, with an average value of $19.88 per kilogram. Production methods by chemical synthesis are outlined in this chapter for ascorbic acid and riboflavin, and in Chap. 4 for riboflavin as a fermentation product. Remington[48] gives procedures for the manufacture and properties of the other vitamins.

ISOLATES FROM PLANTS OR ANIMALS

Although many important pharmaceutical products result from chemical engineering-directed life processes, fermentations, and syntheses, there are some important medicaments whose sole or competitive source is through isolation from plants and animals. Quinine is made by extraction from cinchona bark. Morphine and codeine are isolated from opium, theobromine from waste cacao shells, and caffeine from the decaffeinizing of coffee or from waste tea. Insulin is isolated from pancreas.

RESERPINE USP. Reserpine is one of the alkaloids widely employed for its tranquilizing effect upon the cardiovascular and central nervous systems and as an adjunct in psychotherapy. It is isolated by a nonaqueous solvent process, using, for example, boiling methanol extraction of the African root *Rauwolfia vomitoria*. Naturally, these extractions are carried out under countercurrent methods, details of which are in a flowchart published in *Chemical Engineering*.[49] The methanol extracts are concentrated and acidified with 15% acetic acid and then treated with petroleum naphtha to remove impurities. Extraction is made using ethylene dichloride. The solvent is neutralized with dilute sodium carbonate, evaporated to drive off the ethylene dichloride, and further evaporated to crystallize the crude reserpine crystals. These are further crystallized.

INSULIN INJECTION AND INSULIN ZINC SUSPENSION USP. Insulin, a hormone, plays a key role in catalyzing the processes by which glucose (carbohydrates) furnishes energy or is stored in the body as glycogen or fat. The absence of insulin not only interrupts these processes, but produces depression of essential functions and, in extreme cases, even death. Its isolation and purification were started in April 1922, by Lilly, following its discovery by Banting and Best.

The structure of insulin was elucidated by Sanger and others in 1945 to 1953[50]. Insulin is isolated from the pancreas of beef or hogs and was one of the first proteins to be obtained in crystalline form. In different concentrations and dissociations, the molecular weight of insulin varies between 36,000 and 12,000, and it has up to 51 amino acid units. Insulin protein is characterized by a high sulfur content in the form of cystine. It is unstable in alkaline solution. Insulin is isolated by extraction of minced pancreas with acidified alcohol, followed by purification, as presented in the flowchart in Fig. 40.7 and as outlined in the following description.

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[48]RPS XVI, pp. 945–978.

[49]*Chem. Eng.* 64 (4) 330–333 (1957); Colbert, *Prostagladin Isolation and Synthesis*, Noyes, Park Ridge, N.J., 1973.

[50]Sanger and Thompson, *Biochem. J.* 53 353 (1953).

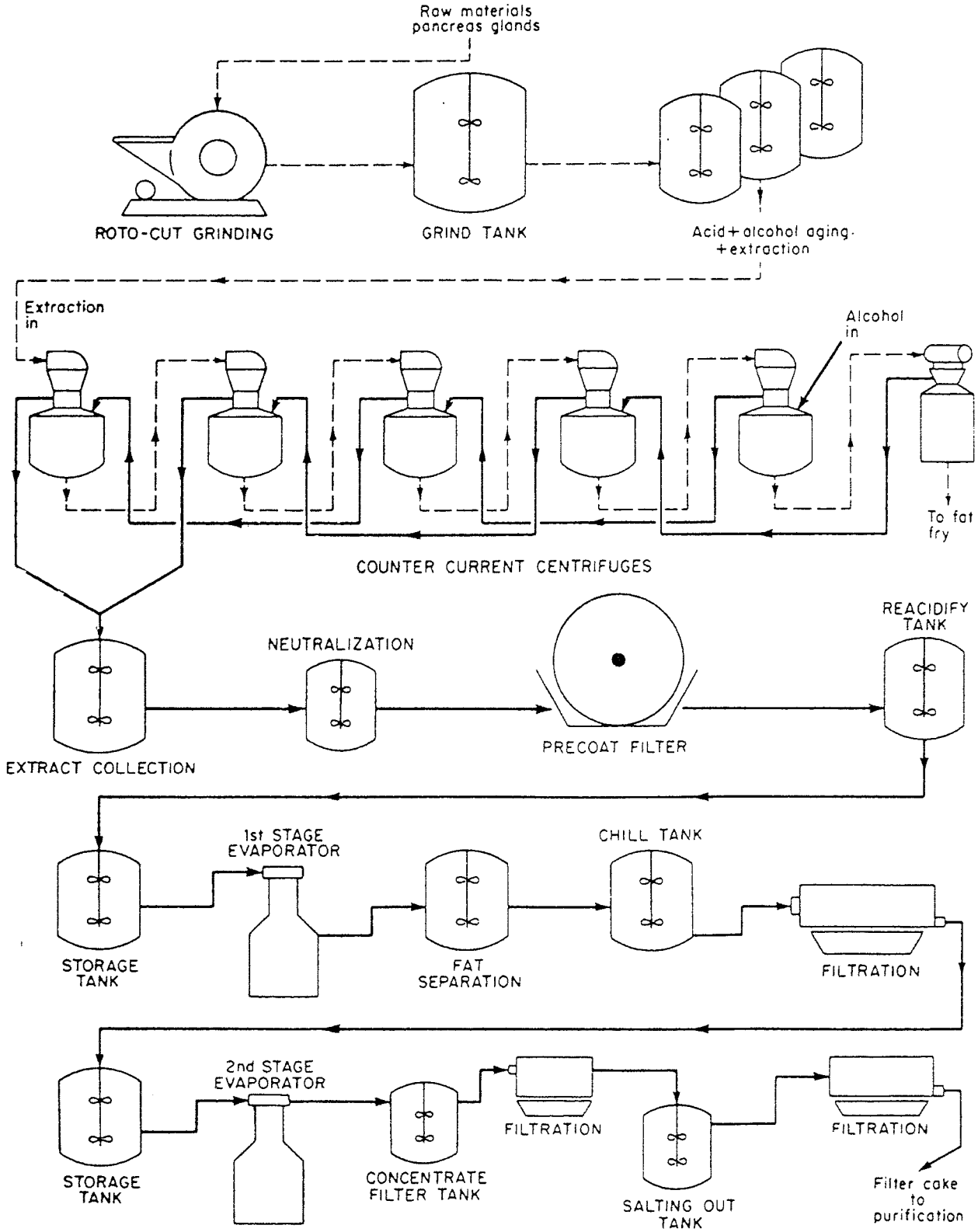

Fig. 6.7. The manufacture of insulin. *(Eli Lilly & Co.)*

Beef and pork pancreas glands are refrigerated at −20°C, rotoground, and the meat slurry is treated with ethyl alcohol after acidification.

With the use of continuous countercurrent extraction in, six continuous centrifuges over extraction tanks, the gland slurry is extracted with acidulated alcohol.

From the sixth centrifuge, the cake is processed in a hot-fat fry tank and discharged

through another centrifuge to separate waste fat from "fried residue," which is drummed and sold.

The crude alcoholic extract is run from two strong extraction-centrifuge units into a collection tank from which the extract is neutralized with ammonia and filter aid added.

In a continuous precoat drum filter, the cake is separated and washed, the clear liquor going to the reacidification tank.

In evaporators, the first stage removes alcohol, with subsequent waste-fat separation. The extract goes to a chill tank, with filter aid added, through a filter press and into the second evaporator.

From the second evaporator, the concentrated extract is filtered and conducted to the first salting-out tank, followed by filter-press filtration with filtrate to sewer and salt cake to purification for the second salting out. A filtration follows with filtrate to sewer.

The second-salting-out product is crystallized twice to furnish Iletin (insulin) crystals.

A method of preparing human insulin[51] is by gene splicing, or a recombinant DNA technique. Two strains of genetically modified bacteria are used to synthesize the two parts (chains A and B) of insulin. The chains are removed from the fermentation mixture and chemically joined together. Swine insulin is similar to human insulin except for a single amino acid building block. A Danish enzyme manufacturer claims to be able to modify swine insulin by replacing one amino acid thus converting it to human type. It is hoped that human insulin will not cause as many side effects in humans as the long-used swine insulin.

COCAINE HYDROCHLORIDE USP.[52] This alkaloid, isolated from *Erythroxylon coca*, formerly obtained from Peruvian coca leaves containing 0.5 to 1%, is now isolated mostly from Java coca leaves containing 1.5 to 2%. In the former, cocaine was the predominating alkaloid and was prepared without great difficulty. However, the Java leaves contain a mixture of cocaine and related alkaloids as the methyl esters of cinnamyl- and benzoyl-ecgonine. The cocaine and by-alkaloids are extracted commercially by alkalizing the ground leaves with a 10% sodium carbonate solution and percolating countercurrently in large steel percolators, using kerosene or toluene. The total alkaloids are extracted from the kerosene or toluene by a process which blows them up with a 5% sulfuric acid solution in tanks. The extracted kerosene or toluene is returned to the percolators. From the sulfuric acid solution, the mixed alkaloids are precipitated by alkalizing with sodium carbonate. The precipitated crude alkaloids are slowly boiled in an 8% sulfuric acid solution for several days to split all the alkaloids to ecgonine. During the splitting, many of the organic acids, like benzoic, are partly volatilized from the kettle with the steam. Those acids that are still suspended and those that crystallize out on cooling are filtered off. The acid aqueous solution of ecgonine is neutralized with potassium carbonate and evaporated. The low-solubility potassium sulfate is filtered hot. Upon cooling, the ecgonine crystallizes out. After drying, it is methylated, using methanol and 92% sulfuric acid, filtered, and washed with alcohol. The methylecgonine sulfate is benzoylated to cocaine in a very vigorous reaction with benzoyl chloride in the presence of anhydrous granular potassium carbonate. The cocaine is extracted from the potassium salts with ether and removed from the ether by sulfuric acid extraction, precipitated with alkali, and crystallized from alcohol. To form the hydrochloride, an alcoholic solution is neutralized with "acid alcohol"

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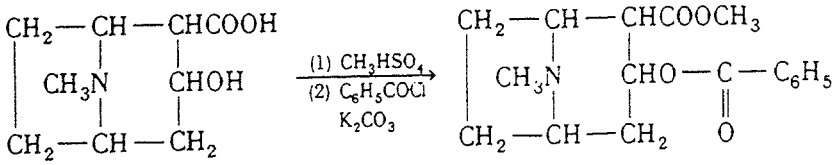
[51]Lilly's Insulin Has a Rival, *Chem. Week* 127 (11) 22 (1980).

[52]RPS XVI, p. 999; ECT, 3d ed., vol. 1, 1979, p. 891; Ashley, *Cocaine, Its History, Uses and Effects*, St. Martins Press, New York, 1975.

(HCl dissolved in absolute C_2H_5OH), and the cocaine HCl crystallized. The synthetic reactions are



Ecgonine $C_9H_{15}NO_3$

Cocaine $C_9H_{13}NO_3(CH_3)\cdot(COC_6H_5)$

In recent years, because of decreased demand, cocaine is obtained as a by-product in the preparation of a decocained extract of *Erythroxylon coca*, this being one of the principal flavors of Coca-Cola and other cola beverages. The procedure is to alkalize the *Erythroxylon coca* and extract *all* the alkaloids with toluene. The decocainized leaf is dried and extracted with sherry wine to give the flavoring extract.

MORPHINE SULFATE USP AND CODEINE PHOSPHATE USP. Remington says that "morphine is one of the most important drugs in the physician's armamentarium, and few would care to practice medicine without it, analgesia being one of the main actions. Codeine is now used to a larger extent than morphine and, while its analgesic action is only one-sixth of morphine, it is employed for its obtunding effect on the excitability of the cough reflex."[53] Morphine (about 11%) and codeine (about 1%) are extracted, along with many of the other alkaloids occurring in opium, by mixing sliced opium balls or crushed dried opium with lime water and removing the alkaloidal contents by countercurrent aqueous techniques. Other solvents are also used, for instance, acetone and acetic acid or acidulated water. The crude morphine alkaloid is precipitated with ammonium chloride, purified by crystallization of one of its inorganic salts from water, and centrifuged; such a crystallization is repeated if necessary. The purified sulfate or hydrochloride is converted into the alkaloid by ammonia precipitation. If a still further purified alkaloid is needed, it can be prepared by crystallization from alcohol. Otherwise the morphine alkaloid is dissolved in water with sulfuric acid and crystallized in large cakes from which the mother liquor is drained and then sucked off. The sulfate is dried and cut into convenient sizes for the manufacturing pharmacist to compound it, make it into tablets, or otherwise facilitate its use by the physicians.

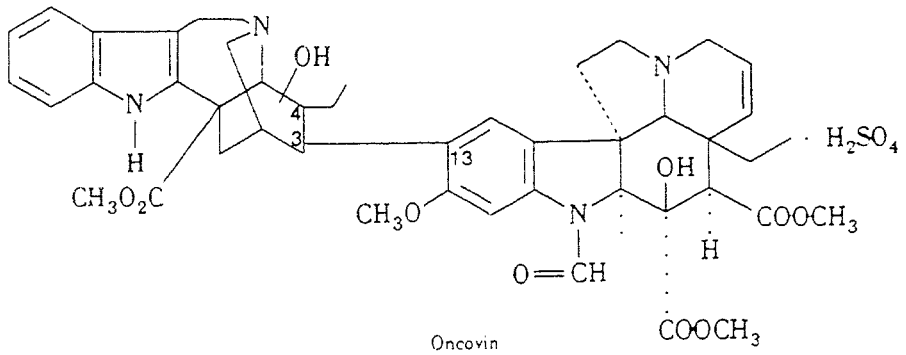
The *codeine* that occurs naturally in the opium is isolated from the aqueous morphine alkaloidal mother liquors by immiscible extraction with a nonaqueous solvent. Dilute sulfuric acid is employed to extract the codeine sulfate from the nonaqueous solvent. This solution is evaporated, crystallized and recrystallized. The alkaloid is precipitated from a sulfate solution by alkali and purified, if necessary, by alcoholic crystallization. It is converted into the phosphate by solution in phosphoric acid, evaporation, crystallization, centrifugation, and drying.

CAFFEINE USP. See caffeine under Alkylation for synthetic production. Much caffeine has been isolated from waste tea, and in recent decades, from the decaffeinization of coffee. The latter process involves extraction at 70°C of the moistened whole coffee bean with an organic solvent, frequently trichloroethylene, reducing the caffeine to about 0.03 from 1.2%. Rotating countercurrent drums are employed. The solvent is drained off, and the beans steamed to remove residual solvent. The beans are dried, roasted, packed, and sold. The extraction sol-

[53]Bentley, *Chemistry of the Morphine Alkaloids*, Oxford Univ. Press, New York, 1954; Small and Lutz, *Chemistry of the Opium Alkaloids*, U.S. Govt. Printing Office, 1932.

vent is evaporated, and the caffeine is hot-water-extracted from the wax, decolorized with carbon, and recrystallized.

VINCA ROSEA ALKALOIDS.[54] An alkaloid derived from *Vinca rosea* Linn has been the basis of two products: *Velban* (vinblastine sulfate, $C_{46}H_{58}N_4O_9 \cdot H_2SO_4$), useful in choriocarcinoma and Hodgkin's disease, and *Oncovin* (vincristine sulfate, $C_{46}H_{54}N_4O_{10} \cdot H_2SO_4$), indicated for acute leukemia in children. It takes 13.5 t tons of periwinkle leaves and 15 weeks of precision chemical processing involving chromatography to yield a single ounce of one of these drugs. Oncovin is probably represented by the formula



Oncovin

SELECTED REFERENCES

Ashley, R.: Cocaine, *Its History, Uses and Effects*, St. Martin's, New York, 1975.

Beckett, A. H.: *Practical Pharmaceutical Chemistry*, 2d ed., 2 vols., Athlone Publ., 1968–1970.

Burger, A.: *Buger's Medicinal Chemistry*, 4th ed., Wiley, New York, 1979–1981.

Dekker, M.: *Pharmaceutical Manufacturing Encyclopedia*, Noyes, Park Ridge, N.J., 1979.

Griffin, R. C. and S. Sacharow: *Drug and Cosmetic Packaging*, Noyes, Park Ridge, N.J., 1975.

Jenkins, G. L.: *Jenkins' Quantitative Pharmaceutical Chemistry*, 7th ed., McGraw-Hill, New York, 1977.

Korolkovas, A.: *Essentials of Medicinal Chemistry*, Wiley, New York, 1976.

Lednicer, D. and L. A. Mitscher: *The Organic Chemistry of Drug Synthesis*, Wiley, New York, 1977.

Osol, A. et al.: *Remington's Pharmaceutical Sciences*, 16th ed., Mack Pub. Co., 1980.

Salerni, O. L. R.: *Natural and Synthetic Organic Medicinal Compounds*, C. V. Mosby, St. Louis, Mo., 1976.

Stenlake, J. B.: *Foundations of Molecular Pharmacology*, Athlone Press, 1979.

USAN and USP Dictionary of Drug Names, United States Pharmacopeial Convention, Inc., Rockville, Md., 1982.

U.S. National Formulary, 15th ed., United States Pharmacopeial Convention, Inc., Rockville, Md., 1980.

U.S. Pharmacopeia, 20th ed., United States Pharmacopeial Convention, Inc., Rockville, Md., 1980.

Wilson, C. O. (ed.): *Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 7th ed., Lippincott, New York, 1977.

Yalkowsky, S. H., A. A. Sinkula, and S. C. Valvani (eds.): *Physical Chemical Properties of Drugs*, Marcel Dekker, New York, 1980.

[54]Sittig, op. cit., p. 645.